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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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EXAMINER	
LEAVITT, MARIA GOMEZ	
ART UNIT	PAPER NUMBER
1633	
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/568,337	WINDISCH ET AL.	
	Examiner	Art Unit	
	Maria Leavitt	1633	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *18 September 2007*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-41* is/are pending in the application.

4a) Of the above claim(s) *9,18 and 29* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-8,10-17,19-28 and 30-41* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *07-05-2006*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Applicant response of 09-18-2007 has been entered. Claims 1-41 are pending. With regard to restriction requirements, Applicant election **without traverse** of the following species is acknowledged: SEQ ID NO. 5 as recited in claims 8, 17 and 28, and a carbohydrate substrate as recited in claim 39. Claims 9, 18, and 29 are withdrawn from further consideration pursuant to 37 CFR1.14(b) as being drawn to nonelected species.

Therefore, claims 1-8, 10-17, 19-28 and 30-41 are currently under examination to which the following grounds of rejection are applicable.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter, which the applicant regards as his invention.

Claim 20 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 20 is rejected under 35 U.S.C. 112, second paragraph, as being incomplete for omitting essential steps, such omission amounting to a gap between the steps. See MPEP § 2172.01. The omitted step is: isolation of the desired polypeptide from the periplasmic space for the production of said polypeptide.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

The present invention is broadly drawn to an expression vector encoding a fusion protein for expression of a heterologous protein, a host cell (e.g., *E. Coli*) comprising said vector and a method for production of the recombinant protein in said host cell, said vector comprising a signal sequence of gac gene (glutaryl 7-aminocephalosporanic acid (ACA) acylase) of *Pseudomonas diminuta* and the polypeptide of interest. Claims 6, 15 and 26 limit the invention to a polynucleotide encoding a signal sequence of *Pseudomonas diminuta* comprising the amino acid sequence of SEQ ID No. 2. Moreover, claims 8, 15 and 28 further limit the invention to a

vector comprising a polynucleotide comprising the promoter and ribosomal biding site of the gac gene of Pseudomonas *diminuta* comprising the nucleotide sequence of SEQ ID No. 5. The specification teaches that the heterologous polypeptide rhIFNα2b (recombinant human Interferon-α2b) is produced in the Escherichia coli K-12 strain W3110 transformed with a plasmid containing an optimized synthetic gene coding for rhIFNα2b. Moreover, the rhIFNα2b is produced under the control of the promoter and Ribosome Binding Site (RBS) of gac gene of *Pseudomonas diminuta* CCM 3987 by fermentation of recombinant *E. coli* K-12. RhIFNα2b is expressed as an N-terminal fusion protein with the signal sequence from the same (gac) gene, directing the protein to the periplasm with concurrent processing (cleaving off) of the signal sequence. Therefore, the fermentation process directly yields mature rhIFNα2b with a primary sequence identical to that of naturally occurring human Interferon α2b (p. 15, Example 1). Additionally, The instant invention contemplates the production of any recombinant polypeptide of interest other than gac gene of *Pseudomonas diminuta* in a prokaryotic host cell (p. 3, paragraph 3) .

Claims 1-3, 6-8, 10-12, 15-17, 19-23, 26-28, 30-41 are rejected under 35 U.S.C. 103(a) as being unpatentable over Peleg et al., (WO 03/004599 A2, Date of Publication 16-Jan-2003) in view or Matsuda et al. (J. of Bateriology, 1985, p. 1222-1228) or Ishii et al., (Journal of Fermentation and Bioengineering , 1994, pp. 591-597) or Kim et al., (Biotechnology Letters, 2001, pp. 1067-1071).

Peleg et al., discloses a method for the production and purification of a fusion polypeptide in *E. Coli*, the method comprising introducing into the bacteria an expression

construct encoding the fusion polypeptide which comprises a TAT-derived peptide and a protein of interest, whereby the TAT derived peptide serves for transport of the fusion polypeptide from the bacterial cytoplasm to the periplasm (Abstract). Moreover, Peleg et al., teaches that transport of proteins through the inner membrane to the periplasmic space requires the inclusion of a signal peptide (p. 3, lines 8-12). Indeed, Peleg et al., exemplifies a construct wherein a bacterial signal sequence in frame with the TAT-derived peptide and protein of interest provides for cleavage of the signal sequence and the TAT-derived sequence, and provides a mature, properly folded and functional protein, readily isolable and purifiable (p. 7, lines 12-16; p. 21, lines 1-5). In preferred embodiments of the invention, Peleg et al., teaches that the vector comprises a polynucleotide harboring a polylinker sequence being operably linked to the signal sequence and a prokaryotic promoter being operably linked to the periplasmic targeting sequence of TAT (p. 11, lines 12-18; p. 20, line 14-17). Additionally, Peleg et al., defines a "signal sequence" a "short (e.g., 15-40) amino acid sequences, which allows proteins to transport through the bacterial inner membrane of he periplasm " (p. 21, lines 23-26). Moreover, Peleg et al., discloses the structure and functionality of bacterial signal peptides for transport of proteins from the cytoplasm to the periplasmic space from mycoplasmas, other gram positive bacteria and *E. coli* (p. 26, lines 27-30 bridging to p. 27, lines 1-19). Peleg et al., teaches at page 25, lines 4-14, plasmids and high copy plasmids such as plasmids from the pUC series (line 5). In relation to the fermentation process, Peleg et al., discloses a multi stage fermentation process comprising a shake-flask step, a pre-culture step and a final culture step (p. 43, lines 5-21). Conditions of fermentation for the production of the polypeptide of interest are disclosed at pages 40-45, including initial incubation temperature at 30°C for 14 hours (p. 41, line 6), fermentation medium containing carbohydrates,

e.g., glucose, fermentation feeding maintained at 4 g/L (p. 43, lines 15-18; p. 43, line 25-26), dissolved oxygen measured to be 30% and maintained by increasing agitation (p. 43, lines 26-29), pH maintained at 7.2 (p. 43, line 22). At page 14, lines 14-30, Peleg et al discloses a list of proteins including interferon (lines 26) to be expressed as a fusion polypeptide in the periplasmic space where processing occurs, providing the mature protein for isolation (p. 42, lines 25-27).

Peleg et al does not disclose a signal sequence of the gac gene of *Pseudomonas diminuta.*

However, at the time the invention was made, Matsuda et al. teaches the nucleotide sequence of the glutaryl (GL) 7-ACA acylase gene from *Pseudomonas sp.* GK16 comprising a nucleotide sequence encoding a 29 amino acids immediately upstream from the small subunit, said 29 aa sequence is disclosed as a signal peptide because of its structure (p. 1226, col. 1 and 2, Fig. 5). Moreover, Matsuda et al. teaches that acylase activity was found in periplasm in the *E .coli* clone, corresponding to previous observations that the enzyme appeared to be periplasmic (p. 1226, col. 1 and 2, Fig. 5). Thus Matsuda et al. clearly teaches that the GL 7-ACA acylase from Pseudomonas sp. GK16 is transported to the periplasmic space in *E. coli.* It is noted that the nucleotide sequence GL 7-ACA acylase gene taught by Matsuda et al. comprises SEQ ID No. 2 (i.e., signal peptide) and SEQ ID NO. 5 (i.e., promoter region and ribosomal biding site as set forth and claimed in the instant invention). Similarly, Kim et al., discloses the gene coding for GL 7-ACA acylase from *Pseudomona diminuta* KAC-1 comprising a putative ribosome-binding site and a signal sequence comprising both SEQ ID No. 2 and SEQ ID NO. 5 as claimed in the instant invention. Likewise, Ishii et al., teaches the GL 7-ACA acylase from *Pseudomona* strain C427 comprising a 27 amino acid signal peptide immediately upstream from the small subunit (p. 592, Fig. 2). Moreover, Ishii et al., discloses that a proportion of active acylase is secreted

into the periplasm in *E. coli* and the remainder is retained in the cytoplasm. Additionally, Ishii et al. teaches that the amount of precursor protein accumulated in the cytoplasm is greatly reduced when the plasmids for the acylase lacking the signal sequence are expressed (abstract), clearly indicating that in systems of high expression in *E. coli,* the signal sequence was required for translocation through the plasma membrane and that without the signal sequence, active acylase accumulates in the cytosol.

Of note, the process of fermentation as encompassed by claim 32 comprising a culture medium with a substrate for more than about 90% of the cultivation time at a substrate concentration lower than the saturation constant of the substrate, accompanied by high levels of dissolved oxygen concentration, which is further accompanied by a steadily decreasing specific growth rate of the bacterial host cells is intrinsic to the process of fermentation in absence of evidence to the contrary.

Therefore, in view of the benefits of producing and isolating a desired protein by using a recombinant fusion protein comprising a signal peptide and the protein of interest, whereby the effective periplasmic targeting sequencing transports the fusion polypeptide form the bacterial cytoplasm to the periplasm as taught by Peleg et al., it would have been *prima facie* obvious for one of ordinary skill in the art, as a matter of design of choice, to modify the fusion protein to introduce into the expression vector a polynucleotide encoding the signal peptide of the gac gene to secrete the heterologous protein into the periplasm of *E. coli*, particularly because Peleg et al., and Matsuda et al. or Ishii et al., or Kim et al., suggest and teach that the introduction of signal sequence of the gac gene of Pseudomona sp. GK16 or *Pseudomona diminuta* or *Pseudomona* sp.

C427 comprising the instantly claimed signal sequence of the gac gene of Pseudomona diminuta is required for translocation and secretion into the periplasm in *E. coli*.

The manipulation of previously identified DNA fragments, cell transformation systems and fermentation conditions for *E. coli* is within the ordinary level of skill in the art of molecular biology. One of ordinary skill in the art at the time the invention was made would had been motivated to combine the Peleg, Matsuda, Ishii and Kim references to improve the efficient and direct production of mature proteins of interest in pure form in a high yield with a reasonable expectation of success.

Claims 4, 5, 13, 14, 24 and 25 are rejected under 35 U.S.C. 103(a) as being unpatentable over Peleg et al., (WO 03/004599 A2, Date of Publication 16-Jan-2003) in view or Matsuda et al. (J. of Bateriology, 1985, p. 1222-1228) or Ishii et al., (Journal of Fermentation and Bioengineering , 1994, pp. 591-597) or Kim et al., (Biotechnology Letters, 2001, pp. 1067-1071) as applied to claims 1-8, 10-17, 19-28, 30-41 above and further in view of Kwon et al., WO 01/057217, Date of publication 9 August 2001).

The combined teachings of Peleg Matsuda, Ishii and Kim have been presented in the paragraphs above. However, none of the references teaches specifically teaches a vector encoding a human interferon alpha in the fusion protein.

At the effective filing of the present application Kwon et al., teaches expression vectors in the form of fusion protein which carry a signal peptide attached to their N-terminal for the secretive production of hIFNα subtypes 2a and 2b (p. 1, lines 6-13; p. 2, lines 27-28; p.3, lines

23-27). Moreover, Kwon et al., successfully exemplified secretion of interferon alpha 2A or 2B in *E. coli* transformants into the periplasm at high productivity (p. 8, lines 1-2).

Therefore, in view of the benefits of producing and isolating a desired protein by using a recombinant fusion protein comprising a signal peptide and the protein of interest, whereby the effective periplasmic targeting sequencing transports the fusion polypeptide form the bacterial cytoplasm to the periplasm as taught by Peleg et al., it would have been *prima facie* obvious for one of ordinary skill in the art, as a matter of design of choice, to modify the fusion protein to introduce into the expression vector a polynucleotide encoding the signal peptide of the gac gene to secrete the heterologous protein into the periplasm of *E. coli*, particularly because Peleg et al., and Matsuda et al. or Ishii et al., or Kim et al., suggest and teach that the introduction of signal sequence of the gac gene of Pseudomona sp. GK16 or *Pseudomona diminuta* or *Pseudomona* sp. C427 comprising the instantly claimed signal sequence of the gac gene of Pseudomona diminuta is required for translocation and secretion into the periplasm in *E. coli.*

Moreover, it would have been *prima facie* obvious for one of ordinary skill in the art, to generate an expression vector as a fusion protein comprising a polynucleotide sequence coding the human interferon alpha 2A or 2B for secretive production, particularly because Kwon et al., successfully exemplified secretion of interferon alpha 2A or 2B in *E. coli* transformants into the periplasm at high productivity. The manipulation of previously identified DNA fragments, cell transformation systems and fermentation conditions for *E. coli* is within the ordinary level of skill in the art of molecular biology. One of ordinary skill in the art at the time the invention was made would had been motivated to combine the Peleg Matsuda, Ishii, Kim and Kwon references

to improve the efficient and direct production of mature interferon alpha 2A and 2B proteins in pure form in a high yield with a reasonable expectation of success.

Conclusion

Claims 1-8, 10-17, 19-28 and 30-41 are not allowable.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Maria Leavitt whose telephone number is 571-272-1085. The examiner can normally be reached on M-F.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Joseph Woitach, Ph.D can be reached on (571) 272-0739. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

To aid in correlating any papers for this application, all further correspondence regarding this application should be directed to Group Art Unit 1633; Central Fax No. (571) 273-8300. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to (571) 272-0547.

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